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TECHNICAL NOTE

On estimating colloid osmotic pressure in pre- and postglomerular plasma in the rat

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Currently in renal physiology much investigative effort is being directed toward an assessment of the role of plasma colloid osmotic pressure (COP) in influencing both the rate of formation of glomerular ultrafiltrate and the rate of reabsorption of this ultrafiltrate by the renal tubules [1–4]. One important consequence of this process of ultrafiltration is that plasma undergoes a progressive increase in its protein concentration (and therefore in COP) in the course of traversing the glomerular capillary network, rising from approximately 6 g/100 ml in the afferent arteriole to values as high as 9 to 11 g/100 ml in the efferent arteriole [1–3, 5, 6]. Thereafter, as a result of the return of reabsorbate to the postglomerular microcirculation, the concentration of protein declines from these high levels to values approaching pre-glomerular concentrations, the exact level being determined primarily by the volume of ultrafiltrate which escapes return to the renal circulation and instead leaves the kidney either as urine or lymph.

At present no method is available for the direct measurement of COP in the small samples of blood which are obtainable from vessels in the postglomerular microcirculation using conventional micropuncture techniques. Instead the current approach to the estimation of COP in pre- and postglomerular blood makes use of 1) the measurement of total protein concentration in systemic and efferent arteriolar blood plasmas [1–3, 5–7], or the measurement of systemic protein concentration and the calculation of postglomerular protein concentration from simultaneous determination of filtration fraction [8], and 2) the application of one of several empirical equations which relate total protein concentration to COP [9]. The equation most frequently employed is that derived by Landis and Pappenheimer [9] for human plasma containing approximately equal concentrations of albumin and globulin (see equation 2 below).

Although the rat is perhaps the species most commonly studied with regard to elucidating the role of COP in influencing filtration and reabsorption [1–8], and although the Landis-Pappenheimer equation is routinely employed in these studies, there is as yet no experimental validation of the applicability of this equation for rat plasmas containing proteins in the wide range of concentrations that obtain in the pre- and postglomerular renal circulation. We therefore undertook to assess the applicability of these equations in the present study.

Methods

Colloid osmotic pressures of rat plasmas containing varying total protein concentrations, and of isotonic saline solutions containing varying concentrations of crystalline bovine serum albumin (Armour Pharmaceutical Co., Los Angeles, Calif. 90063) were measured using a fast response, small aliquot volume, membrane osmometer similar in design and operation to that recently reported by Prather, Brown and Zweifach [10][1]. In this device a semipermeable membrane (PM-30, Amicon Corporation, Lexington, Mass.) separates two chambers of fluid. One chamber is connected to an electronic pressure transducer (P23-Db, Statham Instruments, Inc., Oxnard, Calif.) and is filled with isotonic saline solution having zero colloid osmotic pressure. The other (sample) chamber is filled with the solution in which COP is to be determined. When the test solution containing protein is added to the sample chamber, the difference in transmembrane pressure created by the colloid causes fluid to move from the transducer chamber toward the sample chamber. This creates a negative pressure in the transducer

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[1] Manufactured by Instruments for Physiology and Medicine, Inc., 8082 Lake Adlon Drive, San Diego, Calif. 92119, U.S.A.

chamber which is equal to the COP of the test solution. The signal from the transducer is then processed electronically and displayed on a readout meter. The sample chamber and transducer are surrounded by an electronic heat assembly which is set to maintain a constant environment of 37°C.

Blood samples from rats were collected from the abdominal aorta into lightly heparinized syringes and spun for 10 min at 15,000 rpm. Approximately 0.1 ml of test solution (plasma or albumin) was required for each determination, with equilibration being achieved in approximately one min. Although the volume of the test solution required for measurement of COP can be reduced to 0.05 ml, limitations in design prevent the application of this device to the measurement of COP on the much smaller volumes of plasma obtained directly from surface efferent arterioles (total volumes of 100 to 200 nl). Hence this device does not permit the direct measurement of COP in postglomerular plasma. Total protein concentrations were measured, in duplicate, using the technique of Lowry et al [11].

To examine the reproducibility of the membrane osmometer, isotonic saline solutions containing crystalline bovine serum albumin in final concentrations of 6.0 g/100 ml and 10.0 g/100 ml were measured repeatedly and in the following manner: 0.1 ml of a test solution was deposited into the osmometer well, removed and promptly replaced with a second aliquot of identical volume from the same solution. A reading of osmotic pressure was made at the end of one min, following which the chamber was flushed with isotonic saline. After a brief recovery period (usually less than 30 sec) during which time the osmotic pressure returned to baseline, a subsequent independent determination of COP of the test solution was repeated in similar fashion. Values for 22 such consecutive determinations on the 6 g/100 ml albumin solution ranged from 24.8 to 25.8 mm Hg and averaged 25.3 ± 0.3 SD. For the 10 g/100 ml albumin solution, COP values for 14 consecutive determinations ranged from 56.9 to 58.3 mm Hg and averaged 57.7 ± SD 0.3.

Results

Fig. 1 compares the relationship between bovine serum albumin concentration and measured colloid osmotic pressure (solid circles) with that predicted (solid line) by the empirical Landis-Pappenheimer [9] expression derived for a pure albumin-containing solution:

$$COP_{Albumin} = 2.8\,c + 0.18\,c^2 + 0.012\,c^3 \qquad (1)$$

where c denotes albumin concentration in g/100 ml and COP is given in units of mm Hg. Each of the measured values in Fig. 1 represents the mean of four separate determinations. Note that the fit of the experimental data to the Landis-Pappenheimer prediction is exceedingly close, not only for albumin concentrations at or below 6 g/100 ml, but also for albumin concentrations as high as 11 g/100 ml, the highest concentration tested in this study.

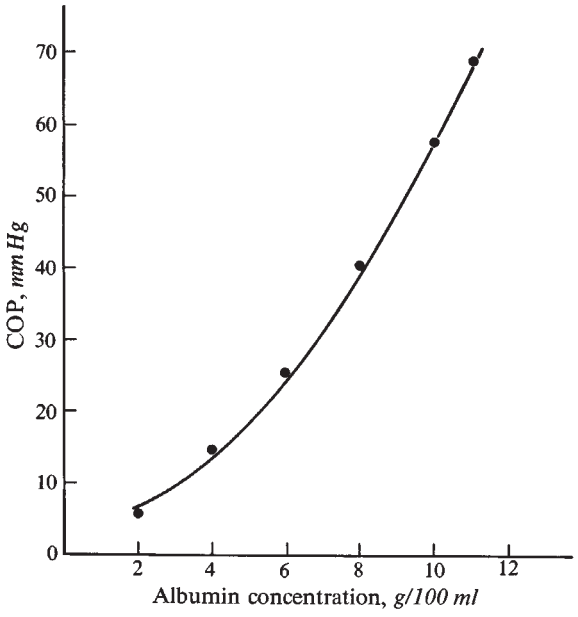

Fig. 1. *A comparison of measured values for colloid osmotic pressure at several concentrations of bovine serum albumin (solid circles) with COP values for these same albumin solutions (solid line) calculated using equation* 1.

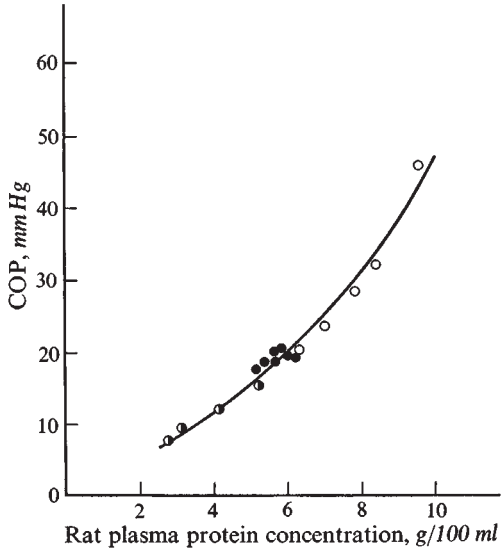

Fig. 2. *A comparison of measured values for colloid osmotic pressure for seven samples of normal rat plasma (solid circles), four diluted "plasma" samples (semi-filled circles) and five concentrated "plasma" samples (open circles) with COP values calculated for these same samples using equation* 2.

Measurements of COP on aliquots of plasma obtained from the abdominal aorta from seven normal adult rats are shown in Fig. 2 (solid circles), plotted as a function of their measured total protein concentrations. The solid line describes the relationship between total protein concen-

tration and COP as derived by Landis and Pappenheimer for human plasma[2]:

$$COP_{Plasma} = 2.1\,c + 0.16\,c^2 + 0.009\,c^3 \qquad (2)$$

where c denotes total protein concentration in g/100 ml and COP is expressed in mm Hg. The paired differences between measured and predicted values for these seven normal rat plasmas ranged from +0.4 to −1.1 mm Hg and averaged −0.2 mm Hg (predicted <measured).

To examine the correspondence between measured and calculated values for rat plasma COP when total protein concentrations were deliberately rendered less than (e. g., as occurs with hemodilution) and greater than systemic levels (as occurs in the early postglomerular microcirculation), aliquots of normal rat plasma were either diluted with varying volumes of isotonic bicarbonate-Ringer's solution (pH 7.4) or concentrated by evaporation[3]. From measurements of total protein concentrations and COP for each diluted or concentrated "plasma", (indicated in Fig. 2 by semi-filled and open circles, respectively) it was possible to compare measured and calculated (equation 2) values for COP at each protein concentration. For the entire population, the agreement between measured and predicted values was uniformly close, with paired differences for the entire group averaging 0.2 mm Hg.

Discussion

We conclude from these observations that the empirical formulae derived by Landis and Pappenheimer [9] accurately predict the protein osmotic pressure properties of solutions of bovine albumin and rat plasma, not only when the protein concentrations of these solutions are in the range normally found in systemic plasma, but also when these concentrations are elevated to levels likely to obtain in the proximal-most portions of the postglomerular microcirculation, or reduced to levels likely to prevail with mild to moderate hemodilution. In addition, this report calls attention to the existence of a rapid-response, small aliquot volume, membrane osmometer which has been found to yield highly reproducible results.

[2] Electrophoretic analysis of rat plasma indicates that the relative concentrations of albumin (approximately 65%) and globulin (35%) are quantitatively nearly identical to that in human plasma [12].

[3] Streams of pure nitrogen gas were allowed to flow over the surface of vials containing normal rat plasma. The degree of evaporation was varied from vial to vial in order to produce plasma samples of increasing total protein concentrations, as shown in Fig. 2.

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